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Sigmoidal relationship between calcitonin and calcium: Studies in normal, parathyroidectomized, and azotemic rats

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Sigmoidal relationship between calcitonin and calcium: Studies in normal, parathyroidectomized, and azotemic rats. Calcitonin secretion is stimulated by acute hypercalcemia. Furthermore, in the rat, the calcemic response to parathyroid hormone (PTH) is decreased by calcitonin stimulation. However, in renal failure, it is not known if an increase in the serum calcium concentration within the physiologic range of serum calcium stimulates calcitonin and whether the increased calcitonin decreases the calcemic response to PTH. In the present study, four groups of pair-fed rats were evaluated: normals (N); parathyroidectomy (PTX); and two groups with renal failure (RF)—basal serum calcium < 8.5 mg/dl (RF_a) and basal serum calcium > 8.5 mg/dl (RF_b). Hypocalcemia was induced by parathyroidectomy or in the RF_a group, by a high phosphate diet. Increases in the serum calcium were produced by a 48 hour infusion of rat 1-34 PTH. In the RF_a and PTX groups, stimulation of calcitonin was observed as the serum calcium increased from hypocalcemia to normal levels of calcium ($P < 0.01$). In all four groups, increasing the serum calcium from normal levels to hypercalcemia increased the serum calcitonin level ($P < 0.05$). The relationship between serum calcitonin and calcium was best expressed as a sigmoidal curve. In the two groups with basal hypocalcemia, PTX and RF_a, the calcitonin-calcium curve was shifted to the left of the N and RF_b groups. In conclusion, 1) the presence of a calcitonin-calcium curve suggests the possibility of a regulatory function for calcitonin during acute increases in the serum calcium; 2) the basal serum calcium concentration may determine the calcium level at which calcitonin is stimulated; and 3) the stimulation of calcitonin in the physiologic range of serum calcium suggests that endogenous calcitonin production may decrease the calcemic response to PTH in renal failure.

Calcitonin, a 32 amino-acid peptide-hormone, is synthesized and secreted by the C-cells of the thyroid gland [1, 2]. Acute hypercalcemia is a recognized stimulus for calcitonin secretion [1, 2]. Moreover, in both humans and rats, an increase in the blood calcium concentration within the physiologic range produces an increase in the serum calcitonin level [3, 4].

Elevation of the serum calcitonin level has been observed in both humans and rats with renal failure [5–8]; this has been attributed to decreased renal clearance of calcitonin [9, 10]. Moreover, in renal failure, the role of calcitonin on calcium regulation is not well defined. In our recent study in rats, we found that stimulation of calcitonin during acute hypercalcemia decreased the calcemic response to parathyroid hormone (PTH) in azotemic rats [11]. This finding suggested that in renal failure, calcitonin may play a role in the observed skeletal resistance to PTH. While hypocalcemia is often observed in renal failure, it is not known whether an increase in serum calcium within the physiologic range stimulates calcitonin secretion. If this were true, then the stimulation of calcitonin may decrease the calcemic response to PTH in renal failure. A better understanding of the serum calcium-calcitonin relationship should help to clarify these questions.

The purpose of this study was to evaluate in renal failure, the relationship between serum calcium and calcitonin, and whether calcitonin is stimulated in the physiologic range of serum calcium. To provide appropriate comparisons, normal and parathyroidectomized rats were also studied.

Methods

Four groups of pair-fed, male Wistar rats weighing between 200 and 300 grams were evaluated. The groups were normals (N, $N = 9$), parathyroidectomy (PTX, $N = 18$), and two groups of rats with renal failure (RF_a and RF_b); the latter two groups were divided according to the basal serum calcium concentration. The RF_a group ($N = 8$) had a basal serum calcium less than 8.5 mg/dl and the RF_b group ($N = 10$) had a basal serum calcium concentration greater than 8.5 mg/dl. In the PTX group, a selective parathyroidectomy was performed with the aid of a dissecting microscope and the adequacy of parathyroid removal was confirmed by a decrease in the serum calcium concentration below 7 mg/dl during a calcium restricted diet (calcium < 0.05%, phosphate 0.8%) for five days. The rats in the PTX group were then returned to a regular diet (calcium 1.2%, phosphate 0.9%). In the two RF groups, renal failure was surgically induced by ligation of two of three main arteries in the hilum of the left kidney followed one week later by a right nephrectomy. Sham operations for renal failure were performed in rats in the N and PTX groups. For any surgical procedure or blood drawing, rats were anesthetized with sodium pentobarbital, 50 mg/kg, administered intraperitoneally. To induce different levels of serum calcium in the two RF groups, rats were either placed on a high phosphate diet (calcium 0.6%, phosphate 1.2%) to induce hypocalcemia or a regular diet to maintain normal levels of serum calcium. Normal rats were fed a

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Table 1. Serum levels of calcium, phosphorus, creatinine, and calcitonin before and after parathyroid hormone infusion

				RF_a	RF_b
				Basal serum calcium	
Serum level		N	PTX	< 8.5 mg/dl	> 8.5 mg/dl
N		9	18	8	10
Calcium	Before	9.70 ± 0.27	6.92 ± 0.36[b]	7.35 ± 0.31[b]	9.75 ± 0.20[c,d]
mg/dl	After	14.15 ± 0.59[a]	11.32 ± 0.59[a,b]	10.35 ± 0.52[a,b]	11.55 ± 0.33[a,b]
Phosphorus[e]	Before	10.27 ± 0.73	12.37 ± 0.83	13.38 ± 1.11[b]	10.19 ± 0.52[d]
mg/dl	After	6.62 ± 0.35[a]	8.89 ± 0.34[a,b]	7.66 ± 1.52[a]	9.67 ± 0.67[b]
Creatinine	Before	0.30 ± 0.03	0.23 ± 0.02	0.84 ± 0.07[b,c]	0.70 ± 0.04[b,c]
mg/dl	After	0.34 ± 0.03	0.27 ± 0.03	0.88 ± 0.08[b,c]	0.75 ± 0.07
Calcitonin	Before	10.5 ± 1.2	10.1 ± 1.0	12.4 ± 1.1[c]	18.2 ± 2.1[b,c,d]
pg/ml	After	39.2 ± 2.5[a]	28.7 ± 3.6[a]	45.7 ± 6.8[a,c]	31.0 ± 4.1[a,d]

Data are mean ± SE.
[a] $P < 0.01$ as compared with before PTH infusion
[b] $P < 0.01$ as compared with N
[c] $P < 0.01$ as compared with PTX
[d] $P < 0.01$ as compared with RF_a
[e] Rats in the N and RF_a groups received a high phosphate diet

high phosphate diet. Except for the calcium restricted diet in the PTX group, all study diets were administered for the two weeks after the right nephrectomy or the sham operation. All rats were fed sixteen grams of food per day.

Two weeks after the induction of renal failure or the sham operation, rat 1-34 PTH (Bachem, Torrance, California, USA) was infused for 48 hours to increase the serum calcium concentration. To vary the increment in serum calcium, the rate of PTH infusion ranged from 0.8 to 2.5 U/100 grams-body wt/hr. The PTH was infused via a subcutaneously implanted miniosmotic pump (model 2001, Alza, Palo Alto, California, USA). Before placement in the miniosmotic pump, the PTH was diluted in 0.9% normal saline with 2% cysteine HCl added to decrease the pH of the solution to 1.5. During the PTH infusion, all rats were maintained on a low calcium and low phosphate diet (calcium < 0.05%, phosphate < 0.2%) to minimize gastrointestinal absorption of calcium and phosphate. On the morning before any procedure or blood drawing, rats were fasted overnight, and were fed after the blood drawing or the completion of the procedure.

Immediately before the PTH infusion, blood was obtained from the tail vein and at sacrifice 48 hours later by cardiac puncture. At both intervals, serum calcium, phosphorus, creatinine, and calcitonin were measured. In addition, in the N and the two RF groups, blood was also obtained from one or two rats at 24 hours. In the PTX and RF_a groups, serum for basal and stimulated calcitonin was not available for measurement at both intervals in all rats. Serum calcium was measured by atomic absorption (Perkin-Elmer, Norwalk, Connecticut, USA), serum phosphorus with a kit for phosphorus (Sigma Chemical Co., St. Louis, Missouri, USA), and serum creatinine with a specific analyzer for creatinine (Creatinine Analyzer 2, Beckman Instruments, Fullerton, California, USA). Serum levels of calcitonin were measured with a commercially available radioimmunoassay (Nichols, San Juan Capistrano, California, USA) that uses a goat antibody raised against synthetic human calcitonin. The validity of this assay in rats has been shown previously [12]. Intraassay and interassay coefficients of variation for the calcitonin assay were 7.5% and 9.5%, respectively. The lower limit of detection was 7 pg/ml.

Biochemical data were analyzed using analysis of variance followed by the Duncan test for multiple comparisons between groups; in addition a paired *t*-test was used to compare values from the same group. A P value < 0.05 was considered statistically significant. All values are expressed as the mean ± standard error (SE).

Results

In Table 1, the serum levels of calcium, phosphorus, creatinine, and calcitonin are shown before and after the PTH infusion. The basal serum calcium level was lower in the PTX group, and by definition in the RF_a group. As expected, the serum creatinine level was higher in the two RF groups. In all groups, the PTH infusion induced a significant increase in serum calcium and calcitonin. Serum phosphorus decreased after the PTH infusion and a reduction in dietary phosphate. As compared with the N group, the other three groups had a lower serum calcium concentration after the PTH infusion. The serum phosphorus level was higher in the PTX and RF_b groups, and the serum creatinine level was unchanged after the PTH infusion.

In Figure 1, the serum level of calcitonin in each group is separated according to the serum calcium concentration: low (< 8.5 mg/dl), normal (8.5 to 11 mg/dl), and high (> 11 mg/dl). Hypocalcemia was not observed in the N and RF_b groups. In all four groups, an increase in the serum calcium concentration from one defined range of serum calcium to the next higher level, produced a significant increase in serum calcitonin. In hypocalcemia (< 8.5 mg/dl), the serum calcitonin level was greater in the RF_a than the PTX group. In the normal range of serum calcium and for a similar mean serum calcium level, the serum calcitonin level was greater in the PTX, RF_a, and RF_b groups than the N group. During hypercalcemia, no significant differences in calcitonin levels were observed between groups. Finally, the serum calcitonin concentration was greater in the basal state in the RF_b than the RF_a group. The respective serum

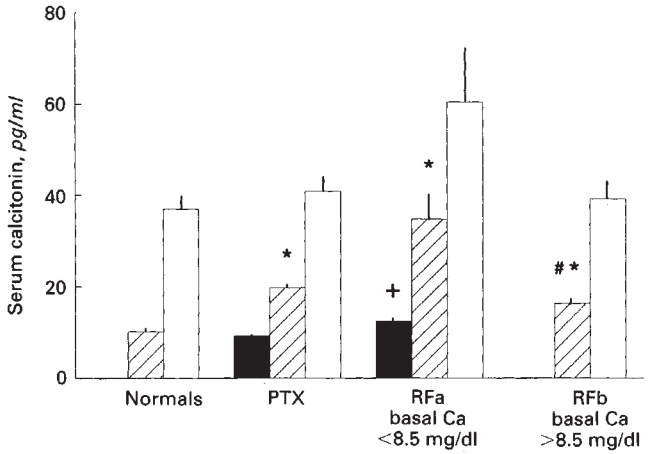

Fig. 1. *The stimulation of serum calcitonin by increases in the serum calcium is shown in the four groups, normals, parathyroidectomy (PTX), and the two renal failure groups, RF_a and RF_b.* The serum calcitonin level is shown for each range of serum calcium, (■) < 8.5 mg/dl, (▨) 8.5 to 11 mg/dl, and (□) > 11 mg/dl. In each group, the serum calcitonin level was significantly greater than the calcitonin level at the previous range of serum calcium. $*P < 0.05$ as compared with normals; $+P < 0.05$ as compared with PTX; $\#P < 0.05$ as compared with RF_a.

calcium concentrations were 7.35 ± .31 (RF_a) versus 9.75 ± .20 (RF_b) mg/dl.

In Figure 2, the relationship between serum calcium and calcitonin is displayed. In Figure 2A, a composite of the four groups is shown and in Figures 2 B through D, the individual groups. As can be observed overall and for each group, a progressive increase in the serum calcium concentration produced an increase in serum calcitonin. Moreover, the relationship appeared to be best represented as a sigmoidal curve. As observed in Figure 2A, the calcitonin-calcium curve in the two groups with hypocalcemia, PTX and RF_a, appeared to be shifted to the left of the other two groups. In renal failure (Fig. 2D), the calcitonin-calcium curve was different between the two RF groups. In RF_a with a lower basal serum calcium level, the calcitonin-calcium curve was positioned to the left of the same curve for the RF_b group; thus, for the same serum calcium concentration, the serum calcitonin concentration was greater in the RF_a than in the RF_b group.

Discussion

To induce hypocalcemia, rats with normal renal function were subjected to parathyroidectomy and rats with renal failure were fed a high phosphate diet. As a result, serum calcitonin levels could be obtained during hypocalcemia and then evaluated as the serum calcium increased. Our results demonstrate that serum calcitonin secretion was stimulated not only by hypercalcemia, but by an increase in the serum calcium during the transition from hypocalcemia to a normal serum calcium level, even when the serum calcium concentration increased within the normal range of serum calcium. However, in at least the N, PTX, and RF_b groups, the increase in serum calcitonin did not parallel the increase in serum calcium inasmuch as the serum calcitonin tended to attain maximal levels at a serum calcium concentration of approximately 12 to 13 mg/dl. Finally, the relationship between serum calcium and calcitonin was best represented as a sigmoidal curve.

Previous studies have indicated that some variation in serum calcitonin levels may occur due to diurnal rhythms. These variations may be induced by feeding [13] or anticipation of feeding [14]. In addition, it has been reported that increases in serum calcitonin induced by feeding are less in male rats [13]. In the present study, rats were fasted overnight and fed after blood was drawn in the morning. Thus, since feeding was regular, blood was drawn at a similar time during the day, and all rats were male, it is likely that the serum calcitonin levels were not significantly affected by diurnal variations.

A decreased calcemic response to a PTH infusion is a well recognized finding in renal failure [15–17]. This phenomenon is believed to be secondary to factors which result from renal failure. These factors include decreased calcitriol levels [15], hyperphosphatemia [16], and down regulation of bone receptors for PTH [17]. In a recent study, we observed that during hypercalcemia induced by a PTH infusion, calcitonin stimulation decreased the calcemic response to PTH [11]. However, our previous study did not address if in renal failure, an increase in serum calcium that did not induce hypercalcemia stimulated calcitonin release. In the present study, we found that calcitonin release was stimulated in renal failure by an increase in the serum calcium even though the serum calcium concentration remained in the normal range. This finding suggests that calcitonin stimulation may modify the calcemic response to PTH in renal failure even in the absence of hypercalcemia.

At similar serum calcium concentrations, differences in basal calcitonin levels were observed between the PTX and RF_a groups during hypocalcemia and between the N and RF_b groups at a serum calcium concentration between 8.5 and 11 mg/dl. The higher calcitonin level in azotemic rats was likely due to decreased renal clearance of calcitonin [5–8]. Finally, renal function did not appear to change during the infusion of PTH. Thus, although it was not possible to measure intact calcitonin, the increase in serum calcitonin, as measured by a carboxy-terminal assay, most likely represented an increase in calcitonin secretion rather than a difference in renal clearance.

For the first time to our knowledge, the calcitonin-calcium relationship has been defined throughout a wide range of serum calcium levels. This type of relationship is well described between PTH and calcium, and is generally represented as a sigmoidal curve [18, 19]. However, as opposed to the PTH-calcium curve in which lowering of the serum calcium stimulates PTH production, elevation of the serum calcium concentration stimulated calcitonin. Our findings in parathyroidectomized, normal, and azotemic rats would suggest that the relationship between serum calcitonin and calcium is best represented as a sigmoidal curve. Another important observation was that hypocalcemia appeared to shift the calcitonin-calcium curve to the left, but did not alter the magnitude of the calcitonin response to hypercalcemia. Thus, for the same serum calcium concentration, the calcitonin level was greater in the normal range of serum calcium when the initial serum calcium concentration was low. This was especially striking in rats with renal failure, and thus raises the possibility that calcitonin may be an important factor modifying the calcemic response to PTH in renal

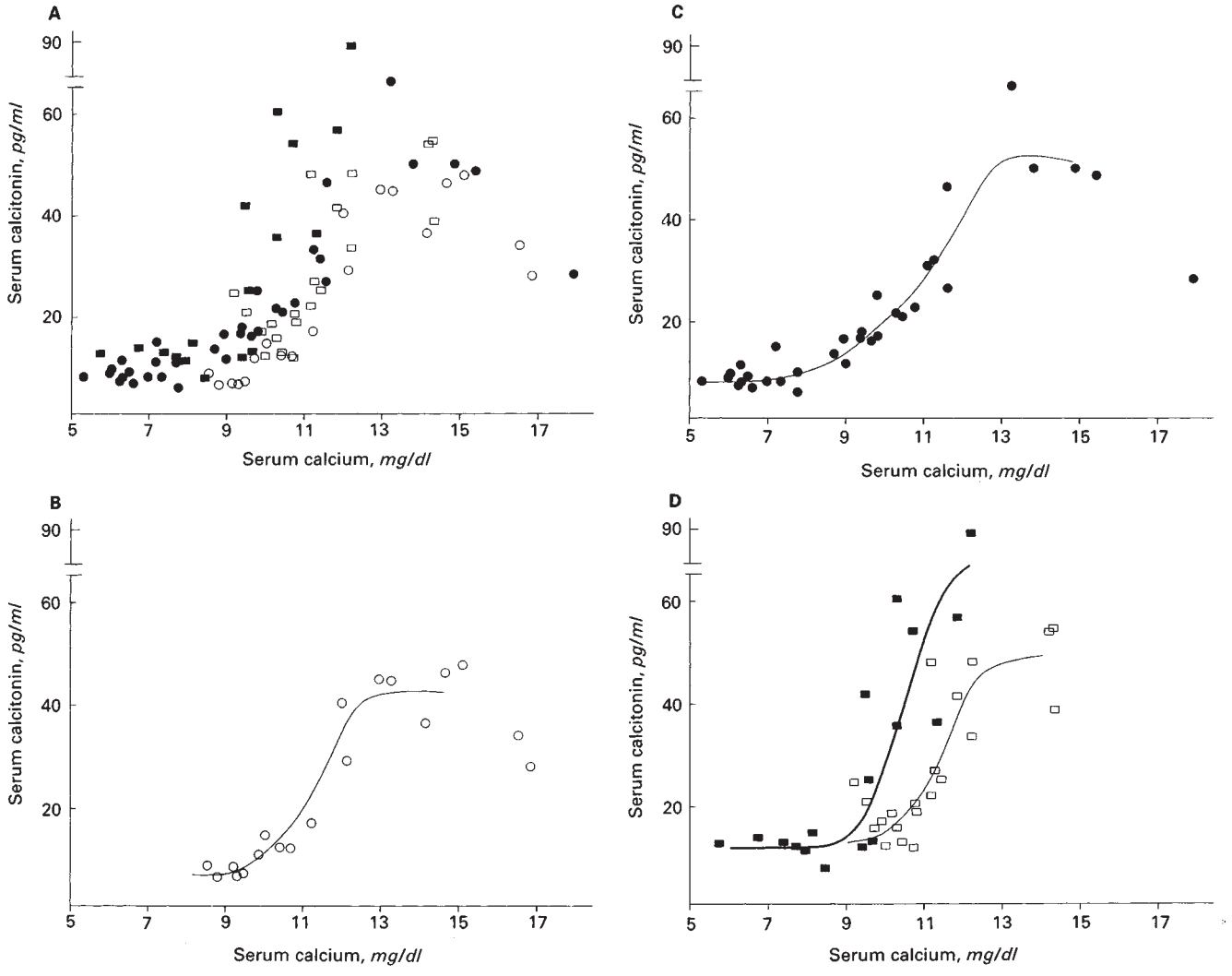

Fig. 2. *The relationship between serum calcitonin and serum calcium is shown.* **A.** Serum levels of calcitonin versus serum levels of calcium in the four groups: (○) N, (●) PTX, (■, < 8.5 mg/dl) RF_a and (□, Ca > 8.5 mg/dl) RF_b. **B.** Serum levels of calcitonin versus serum levels of calcium in the N group (○). **C.** Serum levels of calcitonin versus serum levels of calcium in the PTX group (●). **D.** Serum levels of calcitonin versus serum levels of calcium in the RF_a (■, Ca < 8.5 mg/dl) and RF_b (□, Ca > 8.5 mg/dl) groups.

failure even during hypocalcemia. Further studies, including information in humans, may lead to a better understanding of the physiologic significance of these findings.

The presence of a calcitonin-calcium curve suggests the possibility of a regulatory function for calcitonin. In this study, the serum calcitonin concentration at the ambient serum calcium level was positioned at the base of the slope of the calcitonin-calcium curve. Thus, any increase in the serum calcium elicited a sharp rise in serum calcitonin. This is analogous to the PTH-calcium curve in which the basal PTH level is positioned at the base of the slope and a minimal decrease in the serum calcium concentration produces a marked increase in PTH [18, 20]. However, while hypocalcemia increases PTH messenger RNA synthesis [21, 22], hypercalcemia has not been reported to increase the synthesis of calcitonin messenger RNA [21]. Thus, the increase in calcitonin induced by hypercalcemia may be entirely due to the release of stored hormone. In the three groups in which PTH-induced hypercalcemia exceeded 12 mg/dl, the serum calcitonin level appeared to peak at a serum calcium between 12 and 13 mg/dl, and then either plateau or decline. Thus, if it is true that hypercalcemia does not increase calcitonin synthesis, this observation would suggest that calcitonin stores were depleted. A more remote possibility is that marked hypercalcemia alters the release of calcitonin.

In summary, calcitonin was stimulated as the serum concentration increased from hypocalcemia to normal serum calcium levels. This was observed both in the absence and presence of renal failure. The correlation between serum calcitonin and calcium was best expressed as a sigmoidal curve. In conclusion: 1) the presence of a calcitonin-calcium curve suggests the possibility of a regulatory function for calcitonin during acute increases in the serum calcium concentration; 2) the basal serum calcium level may determine the level of calcium at

which calcitonin is stimulated; and 3) the stimulation of calcitonin in the physiologic range of serum calcium suggests that endogenous calcitonin production may decrease the calcemic response to PTH in renal failure.

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